International Journal of Pediatric Otorhinolaryngology 78 (2014) 1828–1832

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Review Article

Identification of a founder mutation for Pendred syndrome in families from northwest Iran

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ARTICLE INFO

Article history:
Received 25 June 2014
Received in revised form 24 August 2014
Accepted 25 August 2014
Available online 1 September 2014

Keywords:
Pendred syndrome
SLC26A4 gene
Linkage analysis
Haplotype analysis
Founder mutation
Iran

ABSTRACT

Objective: Mutations in the *SLC26A4* gene cause both Pendred syndrome and autosomal recessive nonsyndromic hearing loss (ARNSHL) at the DFNB4 locus. The SLC26A4 mutations vary among different communities. Previous studies have shown that mutations in the SLC26A4 gene are responsible for the more common syndromic hereditary hearing loss in Iran. This study assesses the possibility of a founder mutation for Pendred syndrome in northwest Iran.

Materials and methods: In this study, we performed comprehensive clinical and genetic evaluations in two unrelated families from northwest Iran with nine members affected by hearing loss (HL). After testing short tandem repeat (STR) markers to confirm linkage to the *SLC26A4* locus, we screened the *SLC26A4* gene by Sanger sequencing of all 21 exons, exon–intron boundaries and the promoter region for any causative mutation. We identified the same causative mutation in these two families as we had detected earlier in two other Azeri families from northwest Iran. To investigate the possibility of a founder effect in these four families, we conducted haplotype analysis, and 14 single nucleotide polymorphisms (SNPs) throughout the *SLC26A4* gene were genotyped.

Results: Patients in the two families showed the phenotype of Pendred syndrome. A known frameshift mutation (c.965insA, p.N322Fs7X) in exon 8 was identified in the two families, which was the same mutation that we detected previously in two other Azeri families. The results of haplotype analysis showed that all 15 patients from four families shared the founder mutation. Common haplotypes were not observed in noncarrier members.

Conclusions: Based on the results of our two studies, the c.965insA mutation has only been described in Iranian families from northwest Iran, so there is evidence for a founder mutation originating in this part of Iran.

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http://dx.doi.org/10.1016/j.ijporl.2014.08.035
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1. Introduction

Hearing loss (HL) is a common sensorineural disorder affecting one out of 500 live births [1]. HL is mainly prelingual and recent studies suggest that more than 75% of cases of childhood HL have a genetic origin, such as syndromic forms (25%) and non-syndromic (NS) forms (75%) [1,2]. Mutations in the *SLC26A4* gene have been associated with Pendred syndrome (PS, MIM # 274600) and with DFNB4 non-syndromic hearing loss (NSHL, MIM # 600791). Pendred syndrome (PS) is an autosomal recessive disorder that is associated with sensorineural deafness, congenital and severe to profound temporal bone abnormalities, goiter and iodide organification defects resulting in a positive perchlorate discharge test from the goiter usually in late childhood to early adulthood. In the absence of thyroid dysfunction, patients are considered to be form NSHL DFNB4 [3,4].

The *SLC26A4* gene (NM_000441) encodes pendrin, an iodide–chloride transporter responsible for both NSHL (DFNB4) and PS. Pendrin is expressed in the kidneys, inner ear, and thyroid. In the thyroid, pendrin probably transports iodide ions out of cells and into the inner ear; it is responsible for establishing an appropriate balance between charged particles such as chloride and bicarbonate ions [5]. Mutations in the *SLC26A4* gene probably affect pendrin activity, causing an imbalance of ions and fluid levels in the inner ear. These changes may impair the structure of the inner ear, thus affecting hearing [6]. The *SLC26A4* gene is located on chromosome 7q22.3–7q31.1 and consists of 21 exons [7].

So far, more than 280 mutations in the SLC26A4 gene have been identified in patients with PS and DFNB4 [8,9]. Many of these mutations are common in most people, but some have only been reported in one ethnic group, for example, the c.965insA mutation has only been reported in the Iranian population [10]. In some ethnic populations where a specific mutation is common, mutant alleles may share ancestry (founder mutations) [11–13].

In our study, we report four unrelated Azeri families from northwest Iran with PS, all of whom have an adenine insertion c.965insA at exon 8. From SNP haplotype analysis of mutant alleles in these patients, the c.965insA mutation was determined to be a founder mutation.

2. Materials and methods

2.1. Samples

Four unrelated Azeri families from northwest Iran (Fig. 1) with the syndromic form suggestive of Pendred syndrome were included in this study. Two of the families (Families C and D), including nine individuals with hearing impairment (six males and three females, age range 7–24 years), were referred to our center for molecular diagnosis and the other two families (Families A and B) were from our previous study [10]. Before this study, all participants provided written informed consent according to the protocol approved by the ethics committee of the University of Social Welfare and Rehabilitation Sciences. We performed a comprehensive clinical investigation including functional thyroid tests, the perchlorate discharge test, and serum-free thyroxine and thyrotropin tests to reach a firm diagnosis of PS. Computed tomography (CT) scans of the temporal bone were also performed to check for vestibular aqueduct enlargement.

The perchlorate discharge test involved the oral administration of iodine-131, and the uptake of radioiodide in the thyroid was measured 2 h later. Potassium perchlorate was given orally before the test. The test was considered to be positive when, 1 h after the perchlorate had been administered, the radioiodide in the thyroid had decreased by more than 10% compared to the initial uptake.

Fig. 1. Map of Iran including the locations of the four Azeri families; two families (C, D) from Ardabil and Uremia Provinces, respectively, were the subject of this study, and two families (A, B) from Ardabil Province located in northwestern Iran, participated in our present and previous studies (shown with yellow points). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Peripheral venous blood samples were obtained for analysis of genomic DNA, and genetic evaluations were performed on all patients affected by Pendred syndrome. Each family had at least two affected children, all of whom had severe to profound hearing impairment (90–120 dB), and were negative for *GJB2* and *GJB6* mutations.

2.2. Mutation detection

We used four short tandem repeat (STR) markers (D7S496, D7S523, D7S501 and D7S1817) for homozygosity mapping analysis. After the STR markers had confirmed the linkage to the *SLC26A4* locus, all exons, exon–intron boundaries, and the promoter region of the *SLC26A4* gene were amplified by polymerase chain reaction (PCR) (primer sequences and PCR conditions available upon request) and sequenced in an ABI 3130-Avant DNA analyzer (Applied Biosystems, Foster City, CA, USA).

2.3. Haplotype analysis method

For haplotype analysis, 14 highly polymorphic single nucleotide polymorphism (SNP) markers, at 12 centimorgan intervals surrounding the c.965insA mutation (Chr. 7: 105471384–105471384 to Chr. 7: 116969876–116969876) closely linked to *SLC26A4*, were genotyped (R1–R14) (Fig. 2C). Oligonucleotide primer sequences were obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The amplified PCR products were sequenced directly using an ABI 3130-Avant DNA analyzer (Applied Biosystems). Codon Usage Analyzer software was used to analyze the sequence data.

3. Results

Follow-up studies focused on four large families. Two of them (Families C and D) were included in this study and two others (Families A and B) had already been studied and reported in 2009

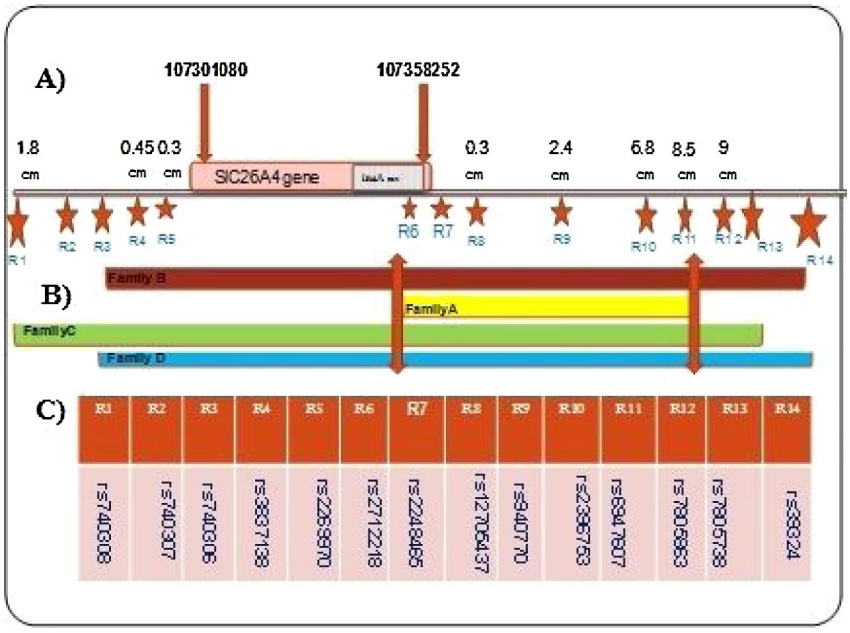

Fig. 2. (A) Genomic organization of the SLC26A4 gene located on chr.7, around the c.965insA mutation and 14 SNP variations that we selected for haplotype analysis. (B) The block from R6 to R11 that was shared between our four families. (C) The names of the 14 selected SNPs.

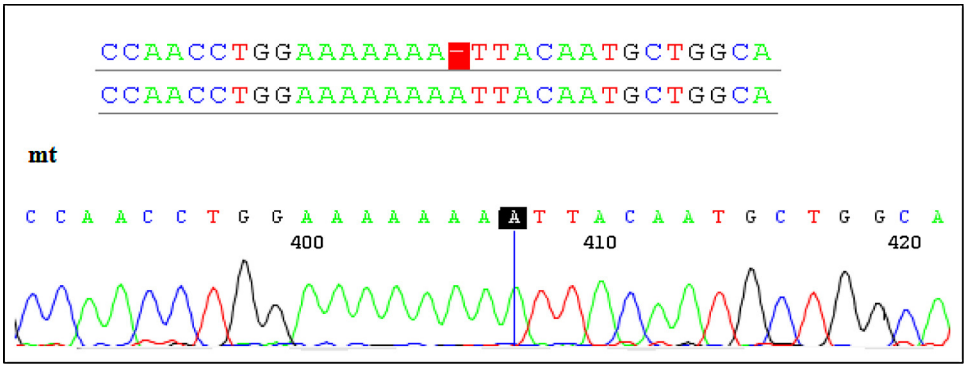

Fig. 3. Sequence electropherogram of the insertion Adenine (c.965insA) in exon 8 (p.N322Fs7X). SLC26A4 gene is shown in the upper panel (the black box in sequencing analysis data illustrates insertion A).

by Kahrizi et al. [10]. This study included 15 individuals affected with Pendred syndrome among 27 siblings from two provinces, Ardabil (Families A, B and C) and Uremia (Family D). In this study, we describe how homozygosity mapping has led to the identification of a frameshift mutation in the *SLC26A4* gene in all of these families.

3.1. Haplotype analysis

Molecular analysis revealed a known frameshift mutation (c.965insA, p.N322fs7X) in exon 8 which led to a stop codon in the *SLC26A4* gene in all four families (15 patients) (Fig. 3). Eight patients in three families (Families A, C and D) were homozygous for this mutation and three patients in one family (Family B) were compound heterozygous for the c.L597S/c.965insA mutation (Table 1) [10].

Because the mutation c.965insA was found in at least one allele in the affected individuals from northwest Iran, we compared the haplotypes of the father, mother, one normal and two affected individuals from each family by genotyping 14 SNP markers at a distance of around 12 centimorgans in the *SLC26A4* gene region. In the four families, c.965insA was associated with a common haplotype for six SNP markers within and close to the putative sulphate transporter gene (PDS) (rs2712218, rs2248465, rs12705437, rs940770, rs2396753 and rs69447607). The four families (A, B, C and D) had the c.965insA mutation, and a common haplotype was only seen in c.965insA carriers, indicating a founder effect for this mutation (Fig. 4).

Table 1
List of families with their origins, variations and genotype–phenotype correlation.

Family	Origin	Description	Variation	Genotype	Phenotype
Family A	Ardabil	Previous study [10]	c.965insA/c.965insA	Homozygous	Pendred syndrome
Family B	Ardabil	Previous study [10]	c.L597/c.965insA	Compound heterozygote	Pendred syndrome
Family C	Ardabil	Current study	c.965insA/c.965insA	Homozygous	Pendred syndrome
Family D	Uremia	Current study	c.965insA/c.965insA	Homozygous	Pendred syndrome

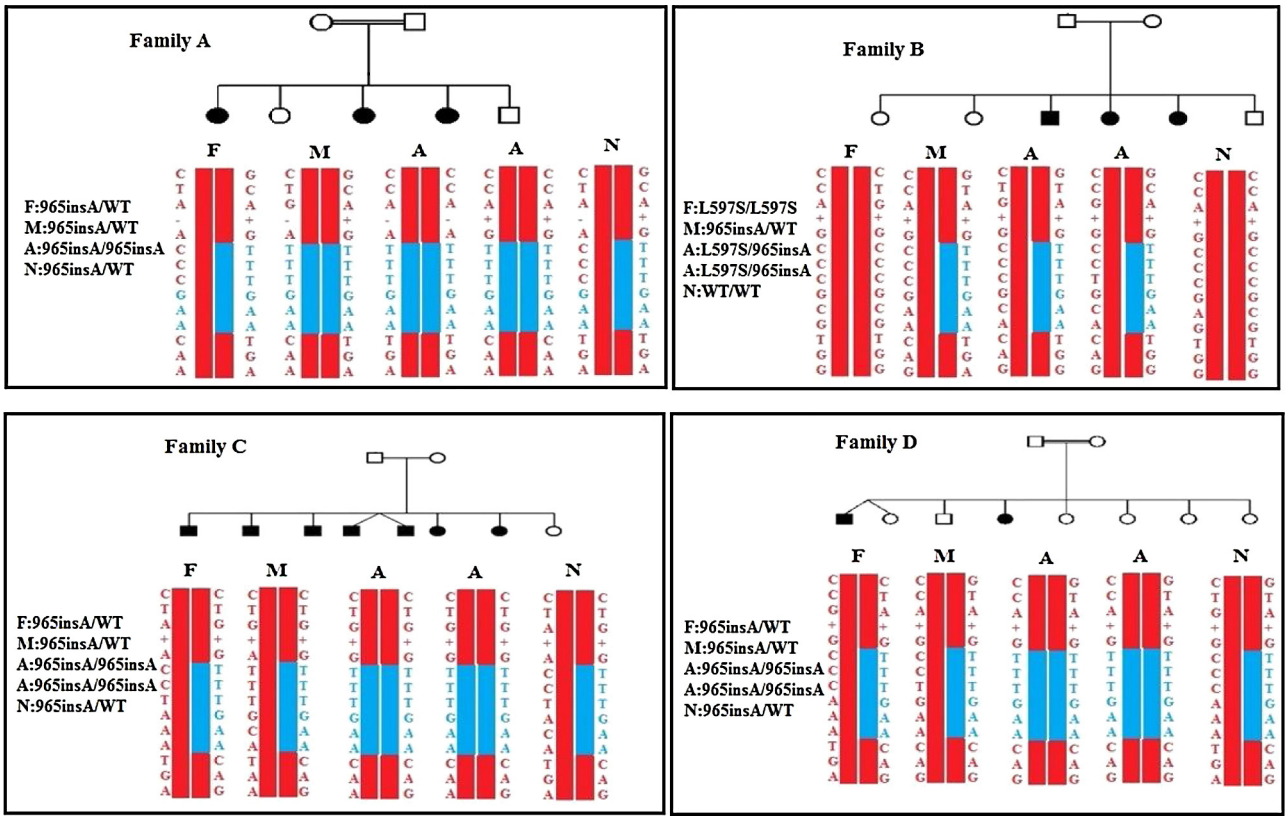

Fig. 4. Genotype and haplotype data for the four families. Each of the four boxes shows: top: family pedigree; left: genotypes; bottom: haplotypes of five individuals in each family (F: father, M: mother, N: normal, and A: two affected). Carriers of c.965insA share a common haplotype (blue color) not observed in noncarrier members, suggesting a founder effect. In Family B, the c.L597S mutation was found in the father and two paternal siblings. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Clinical description

The four families all showed the syndromic phenotype of Pendred syndrome. The pedigree of the families is shown in Fig. 3. All patients had prelingual severe hearing impairment confirmed by audiometric evaluation. The thyroid function test showed thyroid status with diffuse goiter, and temporal bone scans revealed vestibular aqueduct enlargement in three families.

Family A: All three affected females in this family had severe hearing loss (age range 22–28 years). Goiter started after puberty in all affected individuals. They all had hypothyroidism, which was controlled by medication.

Family B: This family had three affected individuals aged between 28 and 34 years. They had moderate to severe hearing impairment and diffuse goiter which had started about 15 years of age in all of them. The thyroid function test in the three affected individuals showed a euthyroid state. Temporal bone scans in one patient showed massive vestibular aqueduct enlargement (class 6).

Family C: In this family, there were seven affected individuals aged between 7 and 24 years with severe prelingual hearing impairment and goiter, which had started after puberty (between 13 and 24 years) in four patients. Temporal bone scans in two patients (one boy and one girl) showed vestibular aqueduct enlargement class 6 (class 1–6).

Family D: This family had two affected individuals aged 18 and 27 years both with profound congenital hearing loss. Their goiter had started at age 14 and 16 years, respectively.

4. Discussion

Mutations in the *SLC26A4* gene encoding for pendrin are responsible for both syndromic and non-syndromic HL. Pendred syndrome is an autosomal recessive disorder associated with hearing loss, goiter and iodide organification defect confirmed by a positive perchlorate discharge test [14].

Currently, more than 280 mutations have been identified in the *SLC26A4* gene in patients with PS and DFNB4 [8]. Many of these mutations are common in most people, but some have only been reported in one ethnic group. Specific mutations identified repeatedly are called founder mutations. For example, the c.965insA mutation had already been reported in two families from west Iran [10–15], and, in the present study, we found this mutation in two other Azeri families. Since the identification of founder mutations in different ethnic groups and their geographical distribution has important implications for the design of mutation screening programs, and mutation in the *SLC26A4* gene is the second most common cause of deafness in the Iranian population [10], we therefore investigated the possibility of a founder mutation in northwest Iran in the current study.

From haplotype analyses, we identified the c.965insA mutation in both mutant alleles in the two individuals affected in Families A, C, and D (they were homozygous mutations) and in one mutant allele in the two individuals affected in Family B (the pathogenic mutation in this family was c.L597S/c.965insA in a compound heterozygous form that was reported earlier [10], and we found this mutation in just one mutant allele in normal individuals and their parents in our four Azeri families.

Our results support the founder mutation theory for the c.965insA mutation. We suggest that, as the c.965insA mutation is more frequent in northwest Iran, it should be included in mutation screen programs in families with suspected syndromic hearing loss indicative of Pendred syndrome.

Conflict of interest

None declared.

Acknowledgments

We would like to thank the patients and their families for participating in this study. Thanks also to Mrs. Khadijeh Jalalvand and Mrs. Sanaz Arjangi from Genetics Research Centre, for their cooperation in this research.

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